

Rice bran oil as a potential resource for biodiesel: A review

Yi-Hsu Ju* and Shaik Ramjan Vali

Department of Chemical Engineering, National Taiwan University of Science and Technology,
43 Sec 4, Keelung Road, Taipei 106-07

Biodiesel (BD) is receiving increased attention as an alternative, non-toxic, biodegradable, and renewable diesel fuel. Exploring new energy resources, such as BD fuel, is of growing importance in recent years. The main concern with BD fuel is its high price. One of the future aims in BD research is on the selection of inexpensive feedstock with high value-added byproducts. Rice bran is a by-product of rice milling that contains 15-23% lipids and a significant amount of nutraceutical compounds. Due to the presence of active lipase in the bran and the lack of economical stabilization methods, most bran is used as livestock feed or boiler fuel and most rice bran oil (RBO) produced is not of edible grade. Thus RBO is relatively an inexpensive raw material for the production of BD. The utilization of by-product such as defatted rice bran for the production of proteins, carbohydrates, phytochemical, and the isolation and purification of value added nutraceutical generated during BD production from RBO are attractive options to lower the cost of BD. Production of BD from RBO can be carried out either via *in situ* esterification, lipase-catalyzed esterification, acid-catalyzed or base-catalyzed reactions. A single step reaction for the conversion of RBO with high free fatty acid content into BD, via acid-catalyzed, base-catalyzed or lipase-catalyzed, fails to attain high conversion in reasonably short time. Pretreatment of crude RBO such as dewaxing/degumming is a crucial step because of its efficient methanolysis. The fatty acid composition of dewaxed/degummed RBO is similar to that of other vegetable oils, which are used as BD feedstock. Various byproducts generated from the rice bran during the production of BD and their applications are also addressed.

Keywords: Defatted rice bran, Methanolysis, γ -Oryzanol, Rice bran oil, Soxhlet extraction, Wax esters

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Introduction

Biodiesel (BD) is obtained through transesterification of vegetable oil, animal fat or waste cooking oil (comprised mainly of acylglycerol and free fatty acid) with monohydric alcohol to give the corresponding monoalkyl ester. The most commonly used alcohol is methanol, and the corresponding fatty acid methyl ester (FAME) is often referred to as BD, which can be used as neat or blend with conventional fossil derived petrodiesel (PD). A blend of BD (20%) in PD is the most popular¹ brand as B20. BD is technically competitive with PD and requires practically no changes in the fuel distribution infrastructure. Although BD faces some technical challenges, such as reducing NO_x exhaust emission, improving cold flow properties, and enhancing oxidative stability, BD has advantages over PD such as the reduction of most exhaust emissions, biodegradability, a higher flash point, greater lubricity, and availability from renewable sources²⁻⁴ (Table 1). BD has higher oxygen content than PD and

its use in diesel engine has shown great reductions in emission of particulate matter (PM), carbon monoxide (CO), sulfur, polyaromatics, hydrocarbons (HC), smoke and noise⁵. In addition, burning of vegetable oil based fuels does not contribute to net atmospheric CO₂ levels because such fuel is made from agricultural materials, which are produced via photosynthetic carbon fixation. Therefore, BD is one of the alternative fuels regarded as environment friendly⁶.

Soybean oil in the United States and rapeseed oil in European countries are the most commonly used BD feedstock. In tropical countries, palm oil and coconut oil are the most common source for BD⁷. Compared to PD, the high cost of BD is a major barricade to its commercialization. BD costs approx one and a half times that of PD depending on the feedstock oil^{8,9}. Majority (70-95%) of BD cost arises from the cost of feedstock oil^{10, 11}. The use of waste-cooking oil is an effective way to reduce the raw material cost^{12, 13}. However, purification is needed before waste-cooking oil can be used as feedstock for BD production¹⁰⁻¹³. During cooking, especially deep-frying, oil is hydrolyzed into free fatty acid (FFA) and degraded by complex chemical reactions. As a result, used cooking

*Author for correspondence

Tel: 886-2-27376612; Fax: 886-2-27376644

E-mail: ju@mail.ntust.edu.tw

Table 1 — Biodiesel specifications

Phsico-chemical properties	Unit	DIN 51606 Specification ^a 6751 limit (1997)	ASTM ^b (2001)
Density	g/cm ³	0.875-0.90	---
Cetane No.	—	Min49	Min 47
Flash point	°C	Min 110	Min 100
Kinetic viscosity at 40°C mm ² /sec	cSt	3.5-5.0	1.9-6.0
Water	% mass	Max 0.03	Max 0.05
Methanol	% mass	< 0.3	---
Free glycerol	% mass	Max 0.02	Max 0.02
Total glycerol	% mass	Max 0.25	Max 0.24
Neutralization No.	mg KOH/g	Max 0.5	Max 0.8
Monoglyceride	% mass	Max 0.8	---
Diglycerides	% mass	Max 0.4	---
Triglycerides	% mass	Max 0.4	---
Total impurities	mg/Kg	Max 20	---
Carbon residue	% mass	Max 0.05	Max 0.05
Ash	% mass	Max 0.02	Max 0.02
Sulfated ash	% mass	Max 0.03	Max 0.02
Phosphorus	% mass	---	Max 0.001

^aDeutsche Norm DIN E 51606, Dieselkraftstoff aus Fettsauremethylesters (FAME), 9, 1997.

^bAnnual Book of ASTM Standards (American Society for Testing & Materials, vol 05.01, WestConshchocken, PA) 1998.

oil contains compounds such as polymers, volatiles, FFA, and other degradation products^{14,15}. Recent report showed that the use of waste cooking oil as BD feed stock produces dioxin and is not on the list of secondary raw materials permitted for recycling or processing as biofuel¹⁶. The cost of BD¹³ from vegetable oil and waste grease are, US\$0.54-0.62/l and US\$0.34-0.42/l, respectively. With pre-tax diesel priced at US\$0.18/l in the USA and US\$0.20 ± 0.24/l in some European countries, BD is thus currently not economically feasible without tax break and/or government subsidies, and more R & D is needed¹³. The use of inexpensive, non-edible feedstock and the utilization of by-products in the BD production may significantly reduce the cost of BD.

Rice Bran Oil: Current Status

Rice (*Oryza sativa* Linn) bran is a byproduct, obtained from the outer layers of the brown (husked) rice kernel during milling to produce polished rice. Whole rice grain comprises (dry weight basis): Endosperm, 70-72; Hull, 20; Bran, 7.0-8.5; and Embryo, 2-3% (Fig. 1). Rice bran comprises pericarp, tegmen (layer covering endosperm), aleurone and sub-aleurone. The rice bran contains oil (15-23%)¹⁷, which is one of the most nutritious oils because of its favorable fatty acid composition vis-à-vis those suggested by expert groups and a unique combination of naturally occurring biologically active and antioxidant compounds such as oryzanol, tocopherols and tocotrienols¹⁸⁻²². Since 1930, the use of rice bran

Table 2 — Composition^a of crude rice bran oil²²

Component	Composition %	Component	Composition %
<i>I Saponifiable lipids</i>	90-96	vii) Phospholipids	3.6-4.8
1 Neutral lipids	80-85	<i>II Unsaponifiable lipids</i>	4.2
i) Triglycerides	66-77	i) 4-Desmethyl sterols ^b	1.8
ii) Diglycerides	2.4-3.6	ii) 4-Monomethyl sterols	0.4
iii) Monoglycerides	4.7-6.2	iii) 4,4'-Dimethylsterols	1.2
iv) Free fatty acids	2-4	iv) Hydrocarbons	0.8
v) Waxes	3-4	v) Tocopherols	0.04
2 Polar lipids		vi) Tocotrienols	0.07
vi) Glycolipids	5.4-6.7		

^aWide variations in the composition of neutral lipids of RBO, in particular the acylglycerides and FFA, occur due to variation of the quality of rice bran

^bA small part of 4-desmethylsterols are esterified with long chain fatty acids and ferulic acid. Nearly all 4,4'-dimethylsterols (triterpene alcohols) are esterified with ferulic acid.

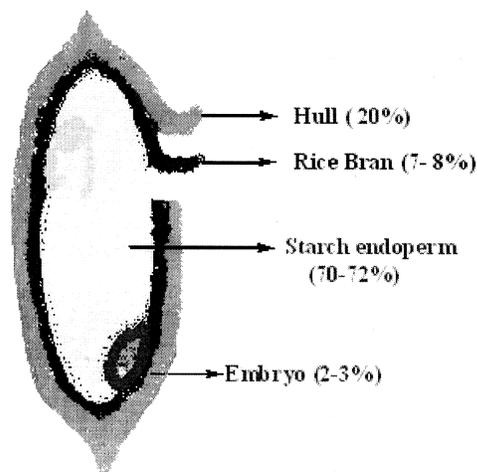


Fig. 1 — Structure of rice kernel

oil (RBO) as edible oil has increased, particularly in Japan, where it is popularly known as “heart oil” as it keeps plasma cholesterol at low level and decreases the risk of cardiovascular diseases. However, crude RBO has been difficult to refine because of its high FFA content, unsaponifiable matter and dark color²³⁻²⁶ (Table 2). Based on the quality of bran, a wide range of variations in the composition of acylglycerol and FFA occurs in most oils extracted from various rice bran samples^{20,27,28}. RBO contains relatively lower content of triacylglycerol compared to other vegetable oils and high contents of partial glycerides, glycolipids, wax esters and unsaponifiable

constituents. The presence of these components modifies the physico-chemical properties of RBO. For example, the viscosity of crude RBO is twice that of common vegetable oils. Partial glycerides, waxes and polar lipids greatly contribute to this effect^{20,29,30}.

Wax in RBO is especially difficult to remove completely. The presence of residual wax imparts haziness to the oil, especially in colder climates. This along with the darker color of the oil, have been responsible for the poor acceptance of RBO by consumers. Another major drawback in producing edible grade RBO from crude oil is its high FFA content. Freshly milled rice bran has a short shelf life because of the decomposition of lipids into FFA by lipases, making it less economical to process into edible oil for human consumption³¹. Rice bran contains several types of lipase that are site specific and cleave the 1,3-site of triacylglycerol (TG)³²⁻³⁴. Owing to this, any delay between rice milling and bran extraction promotes the hydrolysis of oil and results in the development of high FFA. Apart from lipases, rice bran contains amylases, catalase, ascorbic acid oxidase, cytochrome oxidase, lipoxigenases, polyphenol oxidases, dehydrogenase, and esterase and some of them cause deleterious effect of oxidative rancidity in the bran^{28,32-34}. Lipase in rice bran adversely affects the storage quality and the subsequent industrial use of the bran. The enzyme becomes active right after milling and the rate of FFA development (5-7%/d) depending on environmental

conditions. Up to 70 percent FFA can occur after one month of bran storage. Refining of crude RBO (>10% FFA) is uneconomical. FFA (<5%) is desirable in the crude oil for economically producing quality RBO by chemical refining³⁵. Because refining loss is 2-3 times the FFA content, as the FFA content in crude RBO increases, the loss of neutral oil production increases rapidly during edible oil refining³⁶. In addition, chemical refining also produces large volume of environmentally polluting acid water generated during splitting of soapstock. Therefore, inactivation of enzymes in bran is utmost important prior to oil extraction.

The process of hydrolytic rancidity development can be avoided either by rapid oil extraction or stabilization of bran. Enochian *et al*³⁵ analyzed the operational and financial feasibility of using extrusion cooker for rice bran stabilization. In the extrusion cooker, added water, injected steam, or external heat may be required. Bran is held at 125-130°C for a few sec, then at 97-99°C for 3 min prior to cooling to room temperature³⁶. Ramezanzadeh *et al*^{33,37} conducted a series of experiments on the stabilization of rice bran by microwave heating. Stabilization of rice bran by ohmic heating was introduced by Lakkakula *et al*³⁸. Chemical methods to stabilize rice bran were also studied³⁹. Additional production cost is needed if stabilization of bran is practiced. FFA (6-8%) has been found even in oil that was extracted from the bran immediately after the milling of rice. The rapid increase of FFA in the bran has been recognized as a serious problem for the RBO industry. At present, rice bran is mostly used as animal feed and as boiler fuel^{40,41}.

RBO for BD Production

RBO is not a common source of edible oil compared to other traditional cereal or seed sources such as corn, cotton, sunflower, or soybean⁴². The estimated potential yield of crude RBO is about 8 million metric tons if all rice bran produced in the world were to be harnessed for oil extraction⁴³. Full realisation of this potential will help in reducing the cost of BD. Until recently, rice bran was used mostly as animal feed and most of the oil produced is used for industrial applications^{43,44}. One of the best ways for the potential utilization of RBO is the production of FAME (BD). Among the non-conventional oils, RBO is one of the most important in terms of availability. Prices of feedstock and by product meal cake were the two most important factors in the cost of

BD production^{12,13,45}. For example, soybean is a more expensive feedstock for BD than canola, sunflower, rapeseed oils and animal fat because of its low oil content. However, the by-product meal cake of soybean has the highest monetary credit, such that its total cost of BD production is lower than the others. Due to its higher nutritional quality, soybean meal has relatively higher market price than sunflower, rapeseed and canola meals. Crude RBO is a low-cost feedstock for BD production as compared to traditional oils derived from cereal or seed sources. Like soybean meal cake, defatted rice bran is a rich source of protein, carbohydrates and phytochemicals such as phytic acid and myoinositol, which have high commercial value (Fig. 2).

Rice Bran Oilcake

Protein

Defatted rice bran⁴⁶ contains crude protein (14-16%), which has a high lysine content and shows high protein efficiency ratio (PER), high digestibility (< 90%) and is therefore of high nutritional value^{41,47}. Protein contains all essential amino acids⁴⁸. In addition, rice bran is considered as a good source of hypoallergenic proteins and as such, rice bran protein may serve as a suitable ingredient for infant food formulations, thus adding variety to the restricted diets of children with food allergies⁴⁹.

Dietary Fiber

Stabilized rice bran is a good source of both soluble and insoluble dietary fiber (12-15%)⁵⁰, which is almost twice that of oat bran. Studies support dietary soluble fiber as a part of hyperlipidemia treatment^{51,52}. Insoluble fiber functions as a bulking agent, while soluble fiber lowers cholesterol⁵³. Rice bran and its fiber content find a major beneficiary role in the treatment of coronary artery disease⁵⁴. Increased intake of dietary fiber can have beneficial effects against cardiovascular diseases, diverticulosis, diabetes and colon cancer⁵¹⁻⁵⁶. In baked products, the high water-binding capacity of rice bran helps maintain moisture and freshness⁵³.

Phytic Acid

The phytic acid (8.7%)⁵⁷ is comparatively higher than that in other bran and seeds. Phytic acid exhibits strong anticancer activity⁵⁸ and its hexasulfate inhibits the proliferation of Human Immunodeficiency Virus (HIV) *in vitro*⁵⁹. Dietary phytate shows the effects of lowering serum cholesterol and triglycerides in rats⁶⁰. It can potentially be used in dentistry and oral

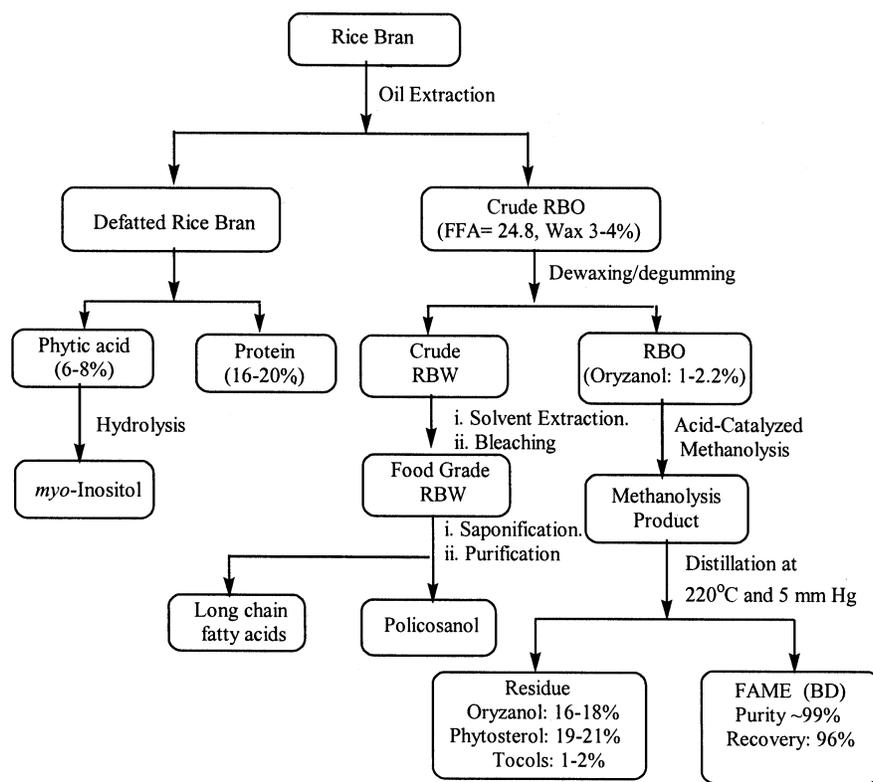


Fig. 2 — Production of biodiesel from rice bran and its byproducts

hygiene⁶³. Considerable work on phytic acid has been carried out by Japanese and Chinese scientists^{20,64-66}.

Myo-Inositol

Hydrolysis²⁰ of phytic acid results in *myo*-inositol, whose deficiency causes alopecia and disturbances in development⁶⁷. *Myo*-inositol analogs act as potential anticancer agents⁶⁸. Dietary inositol also was proved effective in the prevention and treatment of liver cirrhosis and other hepatic diseases⁶⁹. It is used in preventing rough skin and anti-aging cosmetics⁷⁰⁻⁷², and in anti-hypersensitive hair protecting liquid⁷³.

Biologically Active Nutraceuticals from RBO

Rice bran and its oil are a rich source of γ -oryzanol, tocopherols, tocotrienols, wax-ester, policosanol, fatty acid steryl esters and phytosterol^{18-22,43,44}. These compounds find applications in medicinal, pharmaceutical, food and cosmetic industries. Utilization of these compounds lowers the cost of BD and makes it competitive or even cheaper than PD.

Gamma-Oryzanol

RBO reduces LDL-cholesterol and increases HDL-cholesterol. This beneficial activity is believed to arise from the presence of a mixture of ferulate (4-hydroxy-3-methoxy cinnamic acid) esters of

triterpene alcohols and plant sterols¹⁹. Crude RBO contains γ -oryzanol⁴³⁻⁴⁴ (1.1-2.6%). The blood cholesterol lowering activity of RBO in some types of hypercholesteremia was shown to be due to γ -oryzanol^{18,19,74}. Lipid peroxidation prevented in the retina by γ -oryzanol because of its antioxidant property⁷⁵. Moreover, safety assessment clearly indicates that γ -oryzanol possesses no genotoxic and carcinogenic initiation activities^{76,77}. These remarkable beneficial effects of γ -oryzanol on human health have generated global interest in developing commercially viable methods for its isolation from various natural sources and rice bran oil remains the most viable source for its isolation^{43,44}. Studies conducted by Zullaikah *et al*²⁷ showed that γ -oryzanol content of RBO, after a two-step acid-catalyzed methanolysis followed by distillation of FAME, increased (up to 18%) in the black brown colored residue (distilland).

Tocol

Crude RBO contains significant level of tocots (0.2%), of which about 70 percent are tocotrienols^{43,44}. Tocol possesses potent antioxidant⁷⁸ and anti-tumor activity¹⁹, and decreases serum cholesterol and also

hepatic cholesterol synthesis by the suppression of hydroxyl methyl glytaryl coenzyme A reductase. Tocols also play a key role in preventing lipid peroxidation and in delaying pathogenesis of cardiovascular disease, cancer, inflammatory diseases, neurological disorder, cataract, and immunomodulation⁷⁹. Distilland obtained after the distillation of FAME during the production of BD from RBO²⁷ is also a rich source of tocots.

Rice Bran Wax (RBW)

Wax (3-4% on a total lipid basis) is another important product from crude RBO^{43,80}. Like carnauba and other waxes, RBW^{81,82} has potential applications in cosmetic, pharmaceutical, food, polymer, and leather industries. Vali *et al*⁸³ developed a process for the preparation of food grade wax from crude RBW. The properties of purified wax show that it meets specifications of US Federal Food and Drug Administration Act⁸⁴.

Policosanol

Saponification or hydrolysis of RBW results in the formation of long chain fatty acids and policosanols. Saponified wax is mainly a mixture of saturated esters of C22 and C24 fatty acids and C24 to C40 aliphatic alcohols⁸³. The high contents of triacontanol (C30), dotriacontanol (C32) and octacosanol (C28) have been used, with efficacy, as an active ingredient in various pharmaceutical formulations, which show anti-inflammatory activity against ulcers, and/or as protector of gastric and duodenal mucosa⁸⁵. Policosanol possesses blood-lipid lowering effects^{86,87} and improving male sexual activity^{88,89}.

Phytosterols and Fatty Acid Steryl Esters

Steryl ester and phytosterol are well-known ingredients of cosmetic, nutraceutical, and pharmaceutical formulations⁹⁰. RBO is rich (3-5%)^{18,91,92} in phytosterols and fatty acid steryl esters (FASE), which are potent hypocholesterolemic agents^{91,94}. Crude RBO contains phytosterols⁹³ (4,4'-dimethylsterols, 4-monomethylsterols and 4-desmethylsterols), which are present either in free form or as FASE⁹². Both phytosterols and FASE has water-holding property and are widely used as ingredients of cosmetics and bath additives⁹⁴. Phytosteryl ester has been found effective in lowering plasma cholesterol concentration by inhibiting the absorption of cholesterol in human small bowel⁹⁵⁻⁹⁷. Since FASE and oil (triacylglycerol) completely dissolve each other, a lot of attention is being focused on the addition of steryl ester in oil-related foods⁹⁸.

Extraction of Crude RBO

For oil extraction from rice bran, solvent extraction⁹⁹ is most commonly used. Both stabilized and non-stabilized bran can be used for oil extraction. The most widely used solvent for oil extraction is commercial hexane (50-85% *n*-hexane, 15-50% hexane isomers). Hexane has been cleared as a safe solvent for use in the extraction of oil from oilseed or oil-bearing materials under FDA regulations¹⁰⁰. Extraction can be carried out either batch-wise or continuously. In the laboratory, oil extraction can be carried out by the Soxhlet, Butt Type, or Goldfish apparatus¹⁰¹. In commercial operation, bran is palletized, and dried before extraction. An average pellet (6-8 mm diam, 4 mm long, 0.2-0.3 g) is made of bran before subjecting the bran to solvent extraction. Sayre *et al*¹⁰² showed that characteristics of bran and quality of oil from extrusion-stabilized rice bran, where bran changes from fine powder to small flakes by extrusion, remain the same. The extraction characteristics¹⁰³ of rice bran flakes indicated that the flow rate of 58°C hexane through a 60 cm bed was 563-620 l/m²/min. The oil extraction rate was rapid (96% oil was removed within 5 min) and after 1 h of extraction, the residual oil content was only 0.7 per cent (dry basis). Thus, pelletizing extruded bran prior to extraction was not required. The flake form of stabilized bran allows rapid solvent percolation and efficient extraction and increases yield of refined oil. Stabilized bran can be stored for 6 weeks or more without increase in FFA content.

Several studies have reported the use of supercritical carbon dioxide (SC-CO₂) to extract RBO from rice bran^{91,104-106}. Zhao *et al*¹⁰⁴ used a small scale SC-CO₂ plant to extract oil from 20 g of rice bran and obtained a product low in FFA. Ramsay *et al*¹⁰⁵ used a larger SC-CO₂ plant to extract 150 g of rice bran. Under optimum conditions of 30 MPa and 35°C and an extraction time of 5 h, they were able to obtain comparable yields of oil and sterols to those obtained with hexane. Shen *et al*¹⁰⁶ demonstrated feasibility of oil extracting from rice bran using SC-CO₂ at various temperatures and pressures on single-stage pilot equipment. However, SC-CO₂ extraction of oil from rice bran requires more study to make it commercially acceptable and economically viable.

Vegetable Oil as Biofuel

Considerable work has been done by previous researchers on the use of raw vegetable oil as diesel fuel¹⁰⁷. In 1900, Rudolf Diesel first demonstrated the

Table 3 — Typical fatty acid composition^a (%) of crude RBO, dewaxed/degummed RBO and other refined vegetable oils²⁷

Fatty acid	CRBO ^b	D/DRBO ^c	Soybean	Sunflower	Cottonseed	Palm
Lauric	0.2 ± 0.1	n.d.	n.d.	n.d.	n.d.	0.2 ± 0.1
Myristic	0.8 ± 0.1	0.21 ± 0.1	n.d.	n.d.	1.2 ± 0.05	1.11 ± 0.2
Palmitic	17.7 ± 0.53	14.7 ± 0.47	10.4 ± 0.52	6.06 ± 0.25	20.6 ± 1.2	40.20 ± 1.42
Palmitoleic	0.23 ± 0.1	0.26 ± 0.1	0.35 ± 0.1	n.d.	0.8 ± 0.11	0.42 ± 0.1
Stearic	2.2 ± 0.15	1.86 ± 0.22	4.7 ± 0.35	4.8	4.6 ± 0.31	4.5 ± 0.35
Oleic	40.6 ± 0.77	42.2 ± 0.68	24.8 ± 0.66	20.5 ± 0.63	19.54 ± 0.43	43.3 ± 1.33
Linoleic	35.6 ± 0.56	37.8 ± 0.51	52.5 ± 1.52	67.7 ± 1.2	52.5 ± 1.44	9.0 ± 0.37
Linolenic	1.8 ± 0.22	2.39 ± 0.1	6.5 ± 0.33	0.4 ± 0.11	0.23 ± 0.05	1.0 ± 0.23
Arachidic	0.2 ± 0.1	n.d.	0.32 ± 0.1	0.31 ± 0.1	0.31 ± 0.11	0.27 ± 0.14
Behenic	0.3 ± 0.1	0.2 ± 0.1	0.22 ± 0.1	n.d.	0.22 ± 0.08	n.d.
Lignoceric	0.6 ± 0.26	0.3 ± 0.14	0.21 ± 0.1	0.23 ± 0.1	n.d.	n.d.
Saturated/Unsaturated	0.282	0.206	0.188	0.128	0.3685	0.8615

^aMean absolute deviations of three independent determinations.

^bCRBO: Crude rice bran oil

^cD/D RBO: Simultaneously dewaxed/degummed rice bran oil

^dPeak areas less than 0.2% were considered to be negligible and labeled as “not detectable” (ND).

use of vegetable oil as fuel in diesel engine¹⁰⁸. A large amount of research work was also done in the late 1970's and early 1980's in the use of eucalyptus and other oils¹⁰⁹ as diesel fuel. Extensive work on the use of palm oil as a diesel fuel alternative was conducted at Palm Oil Research Institute of Malaysia (PORIM)^{110,111}. The advantages of direct use of raw vegetable oil as diesel fuel are: (1) liquid nature-transportability, (2) heat content (80% of diesel fuel), (3) ready availability, (4) renewability and (5) economic viability¹¹². However, the use of raw vegetable oil as a fuel in compression ignition engines has shown problems of higher viscosity, lower stability and lower volatility¹¹³. The viscosity of vegetable oil is 10-20 times that of diesel fuel¹¹⁴. The content of partial glycerides, wax ester, steryl ester, unsaponifiable matter, polar lipid in the oil and degree of saturation of glycerides are major factors responsible for the high viscosity of raw vegetable oil. The viscosity of crude RBO is 2-3 times that of other vegetable oils. This is due to the presence of high content of wax ester, partial glycerides and unsaponifiable matter^{20,29,30}. To date, no detailed study has been made on the direct use of crude RBO as

biofuel in compression ignition engines. With vegetable oil as the fuel, problems appear only after the engine has been operated on extended periods of time, resulting in severe engine deposit, piston ring sticking, injector coking, thickening of the lubrication, and eventual engine failure^{115,116}.

Deterioration and incomplete combustion are other major problems associated with vegetable oil as biofuel. Vegetable oil contains high amount of polyunsaturated fatty acids (PUFA) such as linoleic and linolenic acids, which contain two and three double bonds, respectively, and are prone to oxidation and autooxidation¹¹⁶. Soybean and sunflower oils contain relatively high levels of PUFA²⁷ (Table 3) and are very susceptible to polymerization and gum formation caused by oxidation during storage or by complex oxidative and thermal polymerization. The gum does not combust completely, resulting in carbon deposits and lubricating oil thickening¹¹⁷. Although crude RBO and refined palm oil contains low level of PUFA compared to other vegetable oils, its high viscosity and saturated fatty acid content are the major drawbacks in direct use as biofuel. Efficient dewaxing/degumming is utmost important if RBO is

to be considered as a biofuel. Modifying vegetable oil through transesterification with monohydric alcohol to form FAME (BD) largely reduces or alleviates these problems^{116,118}.

FAME has low viscosity as compared to that of vegetable oil but maintains similar cetane number and heating value¹¹⁹. The viscosity of methyl or ethyl ester of vegetable oil is approx twice that of diesel fuel. Therefore, FAME can be used directly or as blend with diesel fuel in a diesel engine¹²⁰. Today, the main alternative fuel for diesel engines is a blend of FAME with PD.

Production of BD

Transesterification of oil or fat with alcohol is usually carried out using acid, base or lipase as the catalyst. Three moles of methanol are required theoretically to convert 1 mole of refined oil (100% triacylglyceride) into 3 moles FAME and 1 mole glycerol as follows:

In practice, a higher molar ratio of methanol to oil is required to obtain higher conversion in reasonably short time^{27,121,122}.

Freedman *et al*¹²¹ investigated both acid and base catalyzed transesterifications of soybean oil with butanol and methanol. The methanolysis of triglyceride proceeds via three consecutive reversible reactions as follows:

Noureddini & Zhu¹²² investigated the effect of variations in mixing intensity and temperature on the rate of base-catalyzed transesterification of soybean oil and methanol. A reaction mechanism consists of an initial mass transfer-controlled region followed by a kinetically controlled region with a second-order kinetic mechanism was proposed. Reaction rate constants and activation energies were determined for both forward and reverse reactions.

Base Catalyzed Methanolysis of Oil or Fat

Alkali-catalyzed transesterification is used in the commercial production of BD. The alkali catalyst is usually NaOH or KOH dissolved in methanol. At atmospheric pressure and temperature between 40-65°C, the base-catalyzed reaction can reach 95 percent conversion in 1 h^{123,124}. Knothe *et al*¹²⁵ reported that at optimal conditions (1 wt% KOH catalyst, 69°C and 7:1 alcohol/vegetable oil molar ratio) 97.7 percent conversion can be achieved in 18 min when high purity feedstock was used. For alkali-catalyzed transesterification, starting materials (oil or fat) must be dried (moisture < 0.06%) and free from FFA (< 0.5%); in absence of these parameters,

ester yield was significantly reduced¹²³. The presence of minor amount of FFA and moisture in the reaction mixture produces soap, which lowers the yield of ester and renders the separation of ester and glycerol by making water washing difficult. FFA also consumed the catalyst and reduced catalyst efficiency. Thus, highly refined vegetable oil is required. RBO contains high FFA (6-70%) depending on the quality of rice bran. It is unsuitable as feedstock oil for the production of BD by alkali-catalyzed reactions¹²⁶⁻¹²⁸. Bak *et al*¹²⁹ investigated the production of BD by base-catalyzed transesterification of RBO and alcohol. Experimental parameters studied include molar ratio of RBO/alcohol (1:3, 1:5 and 1:7), concentration of catalyst used (0.5, 1.0 and 1.5 wt%), types of catalysts (sodium methoxide, NaOH and KOH), reaction temperature (30, 45 and 60°C) and types of alcohols (methanol, ethanol and butanol). The conversion of RBO increases with alcohol-mixing ratio and with reaction temperature. Sodium methoxide was the most effective among the catalysts used. The conversion increases with the concentration of catalyst, but the rate of increasing is slow for catalyst concentration over 1.0 wt%. The best conversion (98% in 1 h) was obtained using methanol with sodium methoxide.

Acid Catalyzed Transesterification of Oil

Acid catalyzed transesterification, an alternative chemical method for the production of fatty acid acyl esters from crude RBO or waste cooking oil^{123,126}, can be used when the starting material is low-grade fat or has a high FFA¹³⁰⁻¹³³. In general, acid-catalyzed reactions are sluggish and require high temperature, pressure and longer reaction time^{123,126,130-133}. Although acid catalyzed alcoholysis reaction is slower than alkali catalyzed reaction, it does have the advantage of capability to utilize less expensive low-grade feedstock¹²⁶ containing significant amount of FFA^{126,130-133}. Zullaikah *et al*²⁷ carried out a systematic study on acid-catalyzed reaction (1:10 molar ratio of oil/methanol and 2 wt% of catalyst H₂SO₄, temp 60°C, atmospheric pressure) of methanol with RBO containing wide range of FFA (Fig. 3). The rate of methanolysis and final FAME content in the product depend on the initial FFA content in the RBO. High FAME (96%) can be obtained in 8 h from the RBO (FFA, 76%).

Rapid formation of FAME was observed in the first 2 h due to the rapid methanolysis of FFA. Thereafter, FAME content increases moderately till the end of the

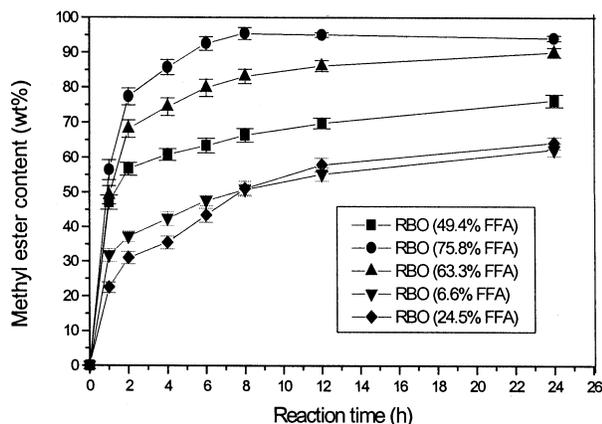


Fig. 3 — Time-course of acid-catalyzed methanolysis of rice bran oil with varying FFA contents

Reaction conditions: Dewaxed/degummed RBO (10 g) was subjected to methanolysis reaction at atmospheric pressure and 60°C using 1:10 molar ratio oil/methanol and 2 wt% sulfuric acid as catalyst.

reaction. For RBO with low FFA (6.6-24.5%), the conversion to FAME is low, and reaches about 60 percent after 24 h due to slow methanolysis of acylglycerides. Analysis of reaction products showed an acid value of 2-3 percent and it remained the same even after 24 h of reaction. This suggests that complete methanolysis of FFA in the RBO is difficult by single step acid catalysis reaction.

A two-step alcoholysis (acid-catalyzed followed by base-catalyzed) has been developed by earlier workers for using oil with high FFA for the production of BD^{127,128,134}. Canakci & Van Gerpen¹²⁷ demonstrated the two step-chemical process using yellow grease (12% FFA) and brown grease (33% FFA). In the first acid-catalyzed alcoholysis step, FFA of the feedstock was reduced (< 1%) and then the transesterification reaction was completed with an alkaline catalyst. Although acid catalyst is effective in the esterification of FFA, there is still a considerable amount of FFA present after the completion of the reaction. Multi-step acid-catalyzed pretreatments have been recommended to reduce FFA to acceptable level (< 0.5%) before base treatment^{27,127,128,135,136}. Zullaikah *et al*²⁷ reported that multi-step acid-catalyzed methanolysis followed by base-catalyzed methanolysis for the production of BD from RBO is not suitable owing to the presence of significant amounts of non-acylglycerides, which forms an emulsion and results in deterioration of the bioactive components during the base treatment¹³⁷.

Goff *et al*¹²⁶ reported a single step acid-catalyzed alcoholysis of soybean oil at elevated temperature (110-120°C) and pressure using 1 wt% of H₂SO₄ and an oil to methanol molar ratio of 1:9 in a sealed reactor. After 26 h, very high FAME (> 99%) in the product was reported. The advantage of this process is that feedstock oil (1-2% FFA) can also be alcoholysed effectively. However, Zullaikah *et al*²⁷ showed that when the same process was applied to dewaxed/degummed RBO with 24.5 and 49.5 percent FFA contents, FAME contents in the product were 62 per cent and 73 percent, respectively. Increase in methanol concentration or catalyst amount had negligible effect on the conversion. For methanolysis of oil with significant amount of FFA, water generated forms another phase which absorbs methanol and H₂SO₄. This water phase probably prohibited the transesterification of TG and the further esterification of residual FFA. Substantial reduction in the conversion during acid-catalyzed alcoholysis of vegetable oil with excessive water (>1 wt%) has been reported^{126-128,136}. Thus, the experimental results described above show that the feedstock containing low FFA (1-3%) as in soybean oil can be efficiently transesterified by single-step acid-catalyzed alcoholysis at elevated temperature and pressure¹²⁶. In addition to the high amounts of catalyst and methanol required, the single-step acid-catalyzed cannot be applied to oil with high FFA such as RBO. Water formed during methanolysis of low grade feedstock with high FFA is the major perpetrator that impede reaction to completion. A few authors^{27,127,128,136} suggested that this could be accomplished by first using a two-step or multi-step acid-catalyzed methanolysis process, removed the polar phase (mixture of water, methanol and glycerol), and subjected the organic phase to base-catalyzed methanolysis.

Zullaikah *et al*²⁷ demonstrated efficient methanolysis of dewaxed/degummed RBO by two-step acid-catalysis to obtain FAME in reasonable short reaction time without damaging the bioactive compound. Before the methanolysis reaction, crude RBO was subjected to dewaxing/degumming³⁰. The first step methanolysis was performed at 60°C using 2 wt% H₂SO₄ and an RBO/methanol molar ratio of 1:5. At a reaction time of 2 h, more than 98 percent FFA were converted into FAME. Polar phase (methanol, water and glycerol) in the reaction mixture was removed, then additional methanol and acid catalyst were added

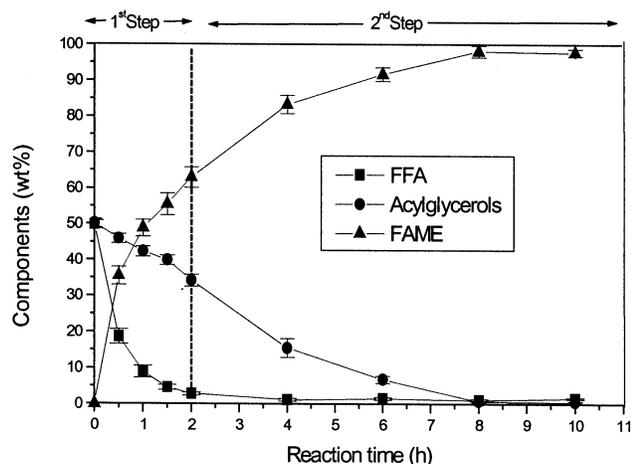


Fig. 4 — Time courses of product composition during two-step acid catalyzed methanolysis of rice bran oil (initial FFA content = 49.8%).

The vertical dotted line indicates the end of the 1st step reaction. FFA: Free fatty acids, Acylglycerols: Mixture of triacylglycerols, diacylglycerols and monoacylglycerols, FAME: Fatty acid methyl ester

to the remaining organic phase and the mixture was subjected to a second methanolysis at 100°C in a sealed reactor. Fig. 4 shows the time-course of variation of product composition in the two-step acid catalyzed methanolysis of RBO (FFA 49.8%). The vertical dotted line indicates the end of the first step and the beginning of the second step reaction. At the end of the first step, the reaction product contained: FAME, 62; FFA 3.2; and acylglycerides, 34.8%. Methanolysis of this product at 100°C resulted in FAME content (> 96%) in the final product with a total reaction time of 8 h. Thus through this two-step acid-catalyzed methanolysis, efficient conversion of low grade RBO into FAME can be accomplished in reasonably short time.

In situ Acid-Catalyzed Transesterification of RBO

Simplification of oil production or esterification process could lower the cost of BD. Hexane contributes to the production of atmospheric smog and to global warming and is classified as a hazardous air pollutant¹³⁸. Since the replacement of lost solvent represents a significant cost to the extraction operation, interest exists in reducing or eliminating the use of hexane in oil extraction. *In situ* production of BD could eliminate the expense associated with solvent extraction¹³⁹. Harrington & D'Arcy-Evans^{140,141} described concept of *in situ* transesterification of sunflower seed oil with acidified methanol and showed significant increase in methyl

ester yields because of the following reasons: (1) By subjecting the whole seed to the esterification process, the lipid content of the hull itself could contribute to the overall yield of esters from the seed; and (2) The esterified lipid, with viscosity and solubility different from those of triglyceride, could prove easier to recover from the solid residue.

Özgül-Yücel & Türkay¹⁴² investigated on *in situ* transesterifications of high-acidity RBO with methanol and ethanol with H₂SO₄ as catalyst and showed that the yield of FAME depends on FFA content of RBO. When 50 g of rice bran, 200 ml of methanol (96%) and 4-5 ml of H₂SO₄ were refluxed for 1 h, about 24 and 86 percent of oil was converted to FAME in rice bran containing 19 and 68 percent FFA, respectively. In another study¹⁴³, the effects of FFA content of RBO, reaction time, temperature, amount of catalyst, rice bran moisture, and amount of methanol on the yield and purity of FAME in *in situ* transesterification were investigated. Effects¹⁴⁴ of FFA content of RBO and the chain length of alcohol (methanol, 96%; ethanol, *n*-propanol, isopropanol, and *n*-butanol, 99.1%) on the conversion of ester in *in situ* esterifications of RBO showed that ethyl ester formation in the ethanol phase increased with increasing FFA content of RBO. Neutral oil solubility in this phase was reduced, resulting in high ethyl ester content. A decrease on the water content of ethanol causes an increase in the neutral oil solubility in ethanol, which promotes ethyl ester formation leading to lower FFA content in the product. The solubility of neutral oil in alcohol had a large effect on the yield and monoester content when high-acidity bran and various monohydroxy alcohols were used. The highest monoester content was obtained with methanol.

All studies on *in situ* esterification of RBO with methanol show efficient esterification of FFA but found that acylglycerides and other nonpolar components were poorly transesterified and mostly remained in the bran. The percentage of oil converted to FAME increases with increase in FFA content of oil. For low-FFA RBO, practically all acylglycerides remain in the bran. It was not possible to esterify all fatty acid and acylglyceride dissolved in alcohol either by increasing the amount of acid catalyst or reaction time and ester fractions isolated from alcohol phase had FFA contents of 6-7 percent¹⁴². Complete extraction of bioactive compounds into methanol phase by *in situ* esterification is also dubious. Thus,

Table 4 — Time course of product composition (wt%)^a of lipase catalyzed methanolysis^b of refined RBO¹⁵⁵

Time h	Novozym-435				IM-60			
	FAME	TG	FFA	MG+DG	FAME	TG	FFA	MG+DG
0	0	98.16	0.8	1.04	0	98.16	0.8	1.84
1	32.72	57.51	1.7	8.06	34.48	51.05	2.4	12.07
2	59.05	34.24	0.6	6.09	60.51	25.19	1.2	13.10
3	80.60	16.14	n d ^c	3.26	71.07	16.34	0.7	11.89
4	89.77	8.48	n d	1.74	80.96	6.14	n d	12.90
5	92.00	6.75	n d	1.25	78.88	7.39	n d	13.73
6	95.84	2.86	n d	1.31	74.30	11.59	n d	14.11
7	98.74	n d	n d	1.27	74.06	11.60	n d	15.34

^aMean absolute deviation of three independent determinations

^bReaction conditions: Methanolysis reactions were conducted as described in experimental procedures under section 2.5

^cNot detectable (GC Peak area < 0.5%)

incomplete esterification of acylglycerides and retaining of bio-active compounds in the bran after *in situ* esterification indicate that production of FAME (BD) by *in situ* transesterification of rice bran is economically not viable at this moment.

PTSA (*p*-toluenesulfonic acid) has been used as chemical catalyst in a process to produce fatty acid acyl esters from RBO and waste cooking oil¹⁴⁵. This process includes removal of impurities from raw fat and oil, followed by a first esterification step in which raw fat or oil reacts with gaseous methanol in presence of PTSA, then a second esterification step in which methanol is mixed with NaOH which functions as a catalyst.

Lipase-Catalyzed Transesterification

One of the disadvantages of both alkali and acid catalyzed methanolysis is that the homogeneous catalyst is removed with glycerol layer after the reaction and it cannot be reused¹⁴⁶. Purification of glycerol as a secondary product is an important step and it is getting more difficult when large amount of inorganic material has to be removed¹⁴⁷. Chemical methods generate substantial amount of effluent and are not environment friendly processes. These problems can be mostly prevented by biocatalyst. Application of lipase in the oleo chemical industry

has become more attractive¹⁴⁸. Using lipase, alcoholysis of low-grade oils such as RBO with high FFA content can be achieved under mild conditions and in reasonable reaction time. Lipase-catalyzed transesterification allows easy recovery of glycerol and avoids disposal of chemical waste.

A plethora of studies have been reported on alcoholysis of vegetable oils and animal fats with various alcohols (primary or secondary, straight or branched-chain) in a batch or continuous process using lipases as the biocatalyst¹⁴⁶⁻¹⁵³. However, only a few studies report on the lipase-catalyzed methanolysis of RBO with various FFA contents for the production of BD. Kamini & Iefugi¹⁵⁴ reported a single step methanolysis of RBO in aqueous medium catalyzed by crude lipase from *Cryptococcus spp.* S-2. About 80 wt% of methyl esters in the product were reported using a oil/methanol molar ratio of 1:4 in the presence of 80 wt.% water and 2000 U of crude lipase with shaking at 160 rpm for 120 h at 30°C. Lai *et al*¹⁵⁵ used refined RBO for the production of BD via lipase-catalyzed methanolysis, and observed a conversion of about 98 percent and 74 percent with Novozym 435 and IM 60 as the catalyst, respectively (Table 4). If pretreated Novozym 435 was used, ester conversion higher than 95 percent can be achieved in 6 h and 1 h

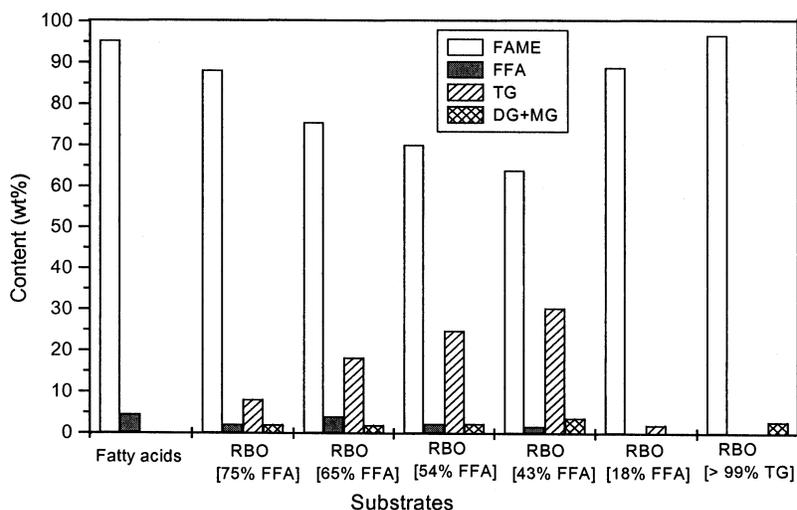


Fig. 5 — Product composition of lipase-catalyzed methanolysis of fatty acids and rice bran oil with varying FFA¹⁵⁵

Reactions were performed with lipase pretreated lipase novozyme 435 for 24 h.

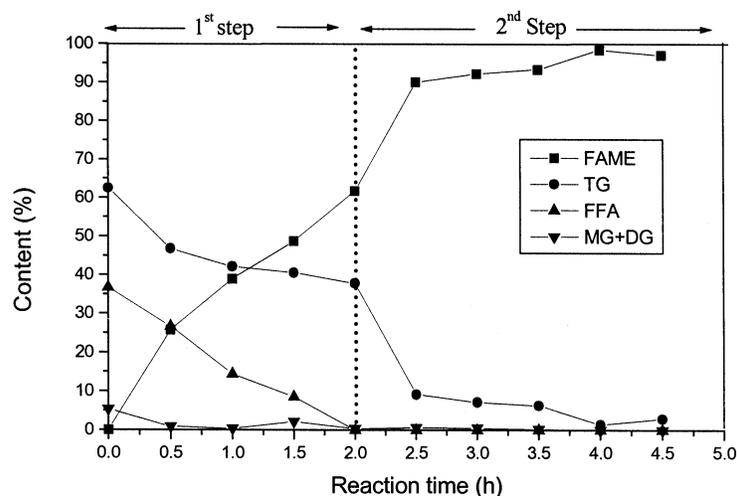


Fig. 6 — Time courses of product composition during two-step lipase catalyzed methanolysis of dewaxed/degummed RBO (FFA, 38.4%)¹⁵⁵

Vertical dotted line indicates the end of first step reaction.

when refined RBO (99% triglycerides) and pure fatty acid (derived from saponified RBO) were used as the substrate, respectively. If RBO contains significant amount of FFA (18-70%), lower conversion is observed and the FAME content in the final reaction product depended on the relative proportion of FFA and acylglycerides present in the starting substrate (Fig. 5). The results show similar trends as obtained in the acid-catalyzed methanolysis of low grade RBO²⁷. Water content markedly reduces the rate of lipase-catalyzed esterification or transesterification^{156,157}. One plausible explanation for this lower conversion in high acidity RBO is that during the lipase-catalyst

reaction co-products such as water, glycerol and methanol had cumulative effect on lipase activity. The solution formed by water and glycerol may possess the right physical property and it may block the access to the active site and act as ester or nucleophile inhibitor¹⁵⁸.

Two-Step Lipase-Catalyzed Enzymatic Process

For efficient conversion of both FFA and acylglycerides to FAME in a short time, Lai *et al*¹⁵⁵ proposed a two-step lipase-catalyzed process (Fig. 6) for methanolysis of RBO with high FFA (20-60%). In the first step, FFA (99%) plus part of acylglycerides were transformed to their corresponding FAME in

2 h. If the reaction were to continue up to 24 h, acylglyceride content will remain approx the same even with the addition of extra methanol. At the end of the first step reaction (2 h), lipase was removed by filtration, washed with hexane (2×5 ml), incubated in tert-butanol for 1 h and then reused in the second step methanolysis reaction. TG content (Fig. 6) can be reduced from 38 percent to 1.5 percent in 2 h in the second step reaction. Thus using two-step methanolysis, rich FAME (98%) can be obtained in a total reaction time of less than 5 h.

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